

THE AUTHENTICITY CONTROL OF VEGETABLE OILS USING THE TOTAL STEROL PROFILE

Francisc Vasile Dulf

University of Agriculture Science and Veterinary Medicine
3-5 Mănăstur Str., 400372 Cluj-Napoca, ROMANIA
e-mail: francisc_dulf@yahoo.com

ABSTRACT

The aim of this study was to develop a rapid and convenient chromatographic method for authenticity control of vegetable oils using the total sterol profile determined by gas-chromatography (GC) with flame ionization detector (FID). Two pure oils, rapeseed and olive oil, processed by minimal technologies (cold pressing) and an adulterated olive oil with 30 % of rapeseed oil were used as sample matrices in the method development. The rapeseed oil can be identified by the presence of brassicasterol, which is characteristic mainly for this oil. In olive oil, this sterol is absent. The concentrations of β -sitosterol, campesterol and brassicasterol as well their ratios can identify the adulteration of virgin olive oil with rapeseed oil. High ratios β -sitosterol / campesterol are specific to virgin olive oil (~ 27.5) while these ratios are low (~ 2) for rapeseed oil or adulterated olive oil. Also the ratio campesterol / brassicasterol can indicate the adulteration. This ratio is low (~ 2) for rapeseed oil and higher (~ 4.8) in extra virgin olive oil. Adulteration of olive oil with rapeseed oil determine the decrease of this ratio. In this work we report three ways to identify the quality and authenticity of olive oil. We found as well markers which indicate if the olive oil was adulterated with rapeseed oil.

1. INTRODUCTION

Adulteration has always been practised and it is carried out for economical purposes (e.g. to increase the bulk volume, to overevaluate a product of inferior quality or to subtract/save expensive ingredients). On the other hand, contaminations may occur accidentally, e.g. in factories, where several oils are produced or used at the same time. These cross-contaminations are usually below 1 – 2 % of the total amount.

In addition, safety problems can be involved in adulterations. Tabuenca J.M. (1981) and De La Paz et al., (1996) reported about the "toxic oil syndrome" (TOS) occurred in the spring of 1981 as an epidemic in Madrid and the north central area of Spain. Over 20,000 cases of illness were documented and over 300 deaths were attributed to TOS. The primary cause for the disease was the consumption of illegally marketed edible oil, adulterated with industrial-grade rapeseed oil containing 2% aniline as denaturant. This example represents one of the most dramatic cases of a hazard to human health associated with an adulteration. An authentic product, whether raw material or a product on the supermarket shelf, is one which strictly complies with the declaration given by the producer in terms of ingredients, natural components, absence of extraneous substances, production technology, geographical and botanical origin, production year and genetic identity.

To assess the authenticity of oils it is fundamental to know, not only the biological origin of seeds, but also the technologies applied, the fat modification techniques used and the chemical composition of the authentic oil(s) and of the potential adulterants (Kamm et al., 2001b).

The fundamental problem for the authenticity assessment of fats and oils is to define one or more parameters within the lipid fraction which allow checking the identity and purity of the specified fat or oil. Ideally, such markers are chemical compounds which are present in the adulterant fat and absent in the original one. However, very often marker substances are not totally absent in the authentic fat but present only in concentrations different from the adulterated product. Therefore, profiles of authentic oils must be compared with the oil to be tested. For the definition of authenticity, the natural variations of the markers, e.g. due to climate, soil and breeding must be taken into account.

Edible oils are derived from a diverse range of plants and their origin is the primary determinant of composition. Thus, the major constituents (generally up to 98 % by mass) in all oils are triacylglycerols. Numerous minor components comprise the remainder. The latter includes free fatty acids, partial acylglycerols, sterols, tocopherols, phenols, stanols, phospholipids, waxes, squalene and other hydrocarbons. Content and composition of these components can vary due to the agronomic and climatic conditions, seed quality, and oil extraction system and refining procedures (Moreda, 2000).

Phytosterols are triterpenes that are important structural components of plant membranes. Most phytosterols contain 28 or 29 carbons and one or two carbon-carbon double bonds, typically one in the sterol nucleus and sometimes a second in the alkyl side chain. Phytostanols are a fully-saturated subgroup of phytosterols (contain no double bonds). Fig. 1 illustrates the structures of three common seed oil sterols, which show small structural variations. The other sterols have the same basic structural design as those illustrated in the Fig. 1.

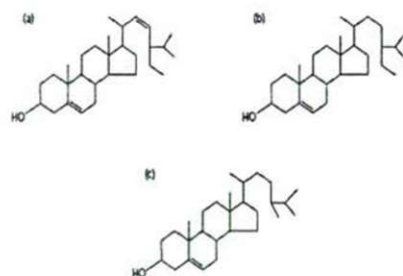


Figure 1. Structures of (a) stigmasterol, (b) β -sitosterol, and (c) campesterol, as main sterols found in plant oils

An important application of sterol fraction analysis is the authenticity determination of olive oils. Specific limits have been assigned to the sterol fraction in olive oil, and if the analysis indicates that the sterol fraction of an oil sample exceeds or fails to meet the limits, the olive oil may have been adulterated with less expensive oil (Hui Y.H., 1996).

The aim of this study was to develop a rapid and convenient chromatographic method for authenticity control of vegetable oils using the total sterol profile determined by gas-chromatography (GC) with flame ionization detector (FID). Two pure oils, rapeseed and olive oil, processed by minimal technologies (cold pressing) and an adulterated olive oil with 30 % of rapeseed oil were used as sample matrices in the method development.

2. MATERIAL AND METHODS

Sampling and Reagents

Two pure, cold pressed vegetable oils, rapeseed and olive oil, and a mixed olive oil with 30 % of rapeseed oil were selected for analysis.

Lipid standards were from Sigma-Aldrich (St. Louis, MO, USA), Merck or Fluka (Buchs, Switzerland). All solvents (analytical-reagent grade or HPLC grade) used were purchased from Merck (Darmstadt, Germany).

Sample preparation for total sterol analysis

The 5 α -cholestane-3 β -ol (2 mg) was used as internal standard. The oils (1.5 \pm 0.01 g) were saponified by refluxing in 70 ml of a 1M KOH ethanol/water (8:2, v/v) solution for 1 h. The refluxed mixture was then transferred into a separatory funnel, and the reflux bottle was washed with 10 ml of water. The unsaponifiables, in the combined solution were then extracted two times: firstly with 15 ml of petroleum ether and secondly with 15 ml of diethyl ether. The ether phase was combined, washed three times with 20 ml 5% NaCl solution, and dried with sodium sulfate overnight. The ether phase was filtered into a evaporation bottle and after was evaporated to dryness (using rotavapor). The residue was transferred in a vial with petroleum ether and stored until derivatisation process.

The sterols were derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) in pyridine (Piironen V., et al., 2003).

GC-FID analysis

The GC separation of sterol TMS ethers was performed using an Rtx-5 fused silica capillary column (5% phenyl / 95% dimethylpolysiloxane, 30 m x 0.25mm i.d., film thickness 0.25 μ m; Restek Corporation, Bellefonte, PA, USA). A SHIMADZU GC-17-A gas-chromatograph equipped with a flame ionization detector (FID) was used. The temperature program was: 5 min at 200 $^{\circ}$ C, 10 $^{\circ}$ C / min to 300 $^{\circ}$ C (hold 20 min). The injection volume was 0.5 μ l (split ratio 1:40). The carrier gas was helium.

The identification of sterols was based on comparison of their retention times (R_t) with data from the literature (Phillips K.M. et al, 2005; Kalo P. et al., 2001;). A mixture of sterol standards (sitosterol 95%, campesterol 98%, stigmasterol 95% and sitostanol 96.7%; Sigma Chemical Co) was studied in the same conditions and the retention times (R_t) were used to assist the peak identification. The sterol concentration were calculated using the area of the internal standard peak.

3. RESULTS AND DISCUSSION

The individual sterol compositions (mg/100g) of the studied two pure vegetable oils (olive and rapeseed) are presented in Fig. 2.

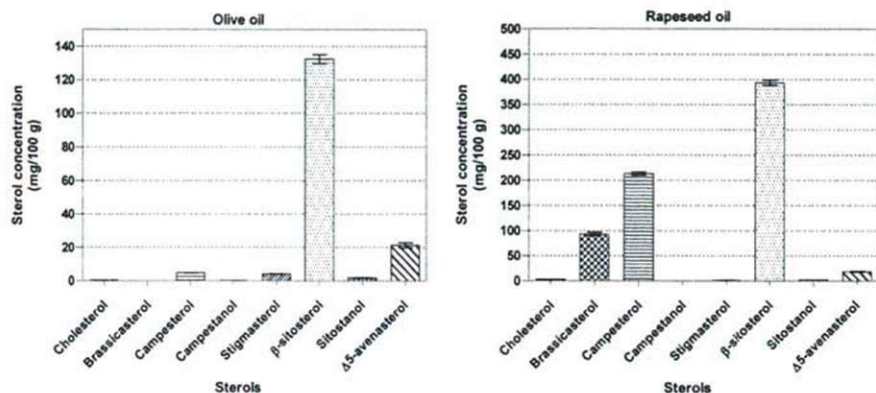


Figure 2. The individual sterol concentrations (mg/100g) of olive and rapeseed oils

The GC-FID chromatogram obtained after analysis of individual sterols in the adulterated olive oil is presented in Fig. 3.

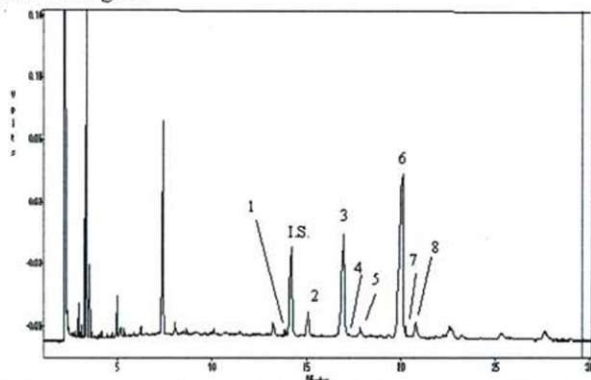


Figure 3. GC-FID chromatogram of olive oil adulterated with rapeseed oil. Peaks are identified as in Table 1.

The individual sterol composition (mg/100g) of the adulterated olive oil is given in Table 1.

Table 1. Sterols contents (mg/100g oil) in adulterated olive oil (extra virgin olive oil +30% rapeseed oil)

Peak nr.	Phytosterol component	Retention times (Rt)	mg / 100 g oil
I.S.	5 α -cholestane-3 β -ol	13.751	
1.	Cholesterol	13.680	1.02
2.	Brassicasterol	15.056	21.31
3.	Campesterol	17.061	96.65
4.	Campestanol	17.217	0.60
5.	Stigmasterol	18.526	4.20
6.	β -Sitosterol	20.272	213.23
7.	Sitostanol	20.601	1.96
8.	Δ 5-Avenasterol	20.771	20.32
Total sterols			359.29

The total sterol content in rapeseed oil was higher (714.55 mg/100g oil) than in olive oil (165.03 mg/100g oil). The rapeseed oil contain higher quantities of β -sitosterol (peak nr. 6), campesterol (peak nr. 3), brassicasterol (peak nr. 2), the ratios (between concentrations) of peaks (6)/ (3) and (3)/ (2) being 1.89 and 2.3 respectively. In extra virgin olive oil the ratios of peaks (6)/ (3) and (3)/ (2) were 27.5 and 4.8 respectively (see Fig.2). After the rapeseed oil was added to olive oil, we observed that the sterol amount in this admixture increased considerable (359.29 mg /100g adulterated olive oil) (Table 1) and the ratios of peaks (6)/ (3) and (3)/ (2) are closer of rapeseed oil.

In the extra virgin olive oil the amount of campesterol was considerable lower (4.60 mg/100 g oil) than in rapeseed oil (212.21 mg/100 g oil). In adulterated olive oil, the amount of this sterol is higher (96.65 mg/100 g adulterated olive oil) than in extra virgin olive oil.

The total sterol profile of olive oil adulterated with rapeseed oil was totally different than the pure olive oil sterol's profile. The presence of the peak nr. 2 (t_R = 15.056), which corresponding to brassicasterol was observed (Fig. 3). So, brassicasterol can be considered a marker for rapeseed oil.

4. CONCLUSIONS

In general, the desmethylsterols (also called sterols) are useful markers to assess authenticity of vegetable oils. Considering that β -sitosterol is the most abundant sterol in the majority of oils, its value has only limited use for the authenticity assessment and differentiation of vegetable oils.

Adulteration of extra virgin olive oil with rapeseed oil can be detected by the presence of brassicasterol. This sterol can be considered the qualitative marker of rapeseed oil, while the concentrations of β -sitosterol, campesterol and brassicasterol as well their ratios can identify the adulteration of virgin olive oil with rapeseed oil. High ratios β -sitosterol/ campesterol are specific to virgin olive oil while these ratios are low for rapeseed oil or adulterated olive oil. Also the ratio campesterol / brassicasterol can indicate the adulteration. Adulterations of olive oil with rapeseed oil determine the decrease of this ratio.

REFERENCES

1. De La Paz M.P., Philen R.M., Borda I.A., Socias J.M.S., De La Camera A.G., Kilbourne E.M., (1996): Toxic oil syndrome: Traceback of the toxic oil and evidence for a point source epidemic. *Food Chem. Toxicol.* 34 251-257 p.
2. Hui Y.H., (1996): Ed., Bailey's Industrial Oil and Fat Products, Vol. 2, Edible Oil and Fat Products: Oils and Oilseeds (John Wiley & Sons, Inc., New York, 5th ed.), pp. 257-259 p.
3. Kalo, P. and Kuuranne, T., (2001): Analysis of free and esterified sterols in fats and oils by flash chromatography, gas chromatography and electrospray tandem mass spectrometry, *Journal of Chromatography A*, 935, 237-248 p.
4. Kamm W., Dionisi F., Hischenhuber C., Engel K.-H., (2001b): Authenticity of fats and oils. *Food Rev. Int.* 17 249-290 p.
5. Moreda W., (2000): Chromatographic analysis of minor constituents in vegetable oils,(Review), *Journal of Chromatography A*, 881, 131-148 p.

6. Phillips, K.M., Ruggio, D.M., Khorassani, M.A., (2005): Analysis of steryl glucosides in foods and dietary supplements by solid phase extraction and gas chromatography, *Journal of Food Lipids*, 12, 124–140 p.
7. Piironen V., Toivo J., Puupponen-Pimia R., Lampi A.-M., (2003): Plant sterols in vegetables, fruits and berries, *J Sci Food Agric*, 83, p.330–337 p.
8. Tabuenca J.M., (1981): Toxic-allergic syndrome caused by ingestion of rapeseed oil denatured with aniline. *Lancet* 12, 567-568 p.